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Developing a species selection index for seed-based ecological restoration in Peninsula Shale Renosterveld, Cape Town

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ABSTRACT

The considerable risk factors associated with ecological restoration make the reintroduction of appropriate species critical to restoration success. In seed-based restoration efforts, using high quality seed is central to good in-field yields; consequently, determining the physiological status of seed prior to large-scale collection and use is important. To gain insight into the suitability of species for use in large-scale seed-based restoration efforts, this study set out to determine the seed viability and seedling emergence of 31 little-studied renosterveld species through laboratory and glasshouse trials, respectively. The outcomes of these seed quality tests were assessed in conjunction with several additional criteria, suggested throughout the literature as relevant to species selection and seed collection, towards determining an overall score, a restoration species index, as a measure of how suitable each species is likely to be in future seed-based restoration efforts. Among the 31 selected species, seedling emergence and to a lesser extent seed viability were variable yet moderate to high for the majority of species. The restoration species index presented here, drawing several considerations into a single, novel approach to species selection, proved useful in this study where the vast majority of species exhibited moderate to high potential.

1. Introduction

World-wide, ecological restoration is a risky endeavour (Crookes et al., 2013) and restoration outcomes are often unpredictable (Clewell and Aronson, 2013). In seed-based restoration efforts, species selection plays a large role in shaping restoration outcomes with respect to species establishment in the short-term and over time the assembly of a community (Clewell and Aronson, 2013; Grime, 1979). Re-introducing the appropriate species has the potential to promote restoration success and lessen some of the risk, yet there is limited guidance, particularly at the community level, pertaining to which species are appropriate for use in seed-based ecological restoration.

Seed-based restoration efforts are reliant on good in-field performance and thus on seed of high quality (Lippitt et al., 1994). Seed viability and seedling emergence are good indicators of potential and likely in-field yields and testing these aspects prior to embarking on large-scale collection and use is important to prevent costly failures (Lippitt et al., 1994). There is, however, a paucity of experimental seed biological-trait data pertaining to renosterveld species.

The merits of determining the potential and likely in-field performance of species are apparent. However, consideration of additional factors is necessary to address potential problem areas commonly associated with seed harvesting and re-introduction. These include the genetic integrity (Mijnsbrugge et al., 2010) and diversity (Way, 2003) of the seed collection; the resilience of the source population to the harmful effects of seed harvesting (Broadhurst et al., 2008); the conservation status of the species (Falk et al., 1996); and, the ease with which one can harvest species (i.e. the effort and thus the cost in relation to the reward).

The source of seed is contentious, yet making use of local provenance seed is recommended for promoting genetic integrity and improving the likelihood of local genotypes establishing and surviving (Bautista et al., 2009; Hufford and Mazer, 2003; Mijnsbrugge et al., 2010). Despite a preference for matching the habitats of the source population and receiving environment (Bautista et al., 2009; Way, 2003), consideration of the proximity of the seed source to the restoration site, despite its limitations, is a practical surrogate for furthering the collection of locally adapted genotypes. Caution against collecting too narrowly within a population is necessary to prevent genetic 'bottlenecks' (Hufford and Mazer, 2003; Kaye, 2001; Mijnsbrugge et al., 2010) and ensure good genetic representation of the population (Way, 2003) and both population size and the extent of area of the source population are useful indicators

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in this regard. Population size is, in addition, a measure of population resilience as the larger the population, the greater its ability to absorb the impacts of seed harvesting (Broadhurst et al., 2008). Assuming adequate population resilience (Kaye, 2001), the conservation status of a species is relevant due to the conservation imperative and benefits associated with establishing a novel population of a species of conservation concern (Falk et al., 1996). Additional aspects of plant abundance and the number of seeds per individual relate to time spent in the field with respect to locating plants and collecting seed and consequently the degree of collection ease, efficiency and cost-effectiveness, practical aspects of paramount relevance to large-scale efforts.

The role of species selection is of central importance to ecological restoration outcomes yet the literature in general fails to advocate seed collection protocols that bring the aspects of genetics, source population resilience, conservation status, collection ease and seed physiology together. Internationally, studies have addressed the selection of species for forest restoration towards overcoming a lack of information pertaining to the component species. Contemporary tropical forest restoration protocols (Elliott et al., 2013), advocate building species selection models on the 'framework species method' established by Goosem and Tucker (1995) and Lamb et al. (1997). Species are commonly selected on the basis of assessing multiple criteria according to numerical scoring systems (Elliott et al., 2013; Knowles and Parrotta, 1995). Some of the criteria identified include aspects of seed physiology, post-establishment performance and species abundance measured by viability, germinability, seedling survival and rarity (Blakesley et al., 2000; Elliott et al., 2003, 2013; Knowles and Parrotta, 1995). Locally, seed-based restoration trials in renosterveld are few (Holmes, 2002a, 2005; Midoko-Iponga, 2004), so efforts have been guided by protocols based on the ecology of fynbos and other fire-adapted shrublands (Holmes and Richardson, 1999). These trials identified appropriate seed source areas and ensured collection from the major growth forms. Since the full representation of species recruits post-fire in fynbos and other fire-driven shrublands (Holmes and Richardson, 1999), seed was collected frequently from as many species as possible (Holmes, 2002a, 2005). However, although within admittedly short timeframes (2 and 3 years), the restoring communities in these studies failed to adequately resemble their respective reference sites (Holmes, 2002a, 2005).

Towards reducing the gulf between restoration outcomes and reference-site benchmarks, we present an index that builds on these previous examples of species selection and draws together considerations relevant to seed collection and re-introduction into a simple, novel approach to addressing the complex underlying issues. In addressing the knowledge gaps this study set out to:

- Determine percent viability and seedling emergence of each of the 31 selected renosterveld species.
- Develop a species selection index for use in seed-based ecological restoration.
- Evaluate each of the selected 31 renosterveld species (in terms of viability, emergence and several additional criteria) for suitability in future restoration efforts.

2. Material and methods

2.1. Study area

Peninsula Shale Renosterveld, one of 29 recognised renosterveld types, occurs within the lowlands of the Cape Floristic Region (Rebelo et al., 2006; von Hase et al., 2003) located at the south western tip of Africa (Manning and Goldblatt, 2012). The Fynbos Biome, comprised of fynbos, renosterveld and western strandveld vegetation complexes (Rebelo et al., 2006), constitutes over 80% of the Cape Floristic Region (Manning and Goldblatt, 2012). The Cape Floristic Region is one of 34 internationally recognised biodiversity hotspots due to extensive habitat loss (Conservation International, 2013) coupled with exceptionally high levels of floristic diversity and endemism (Manning and Goldblatt, 2012). Renosterveld types largely experience a Mediterranean-type climate (Taylor, 1980) and are associated with relatively fertile, clay-rich soils (von Hase et al., 2003). Grazing and fire are important drivers maintaining biodiversity richness in renosterveld (Boucher, 1995; Helme and Rebelo, 2005; Rebelo, 1995), affecting the interplay between grassy and shrubby states (Helme and Rebelo, 2005; Milton, 2007; Rebelo et al., 2006). Peninsula Shale Renosterveld is described as a tall, open shrubland and grassland occurring on gentle to steep gradients and remnants of the vegetation type are situated either side of the Cape Town city bowl on the Cape Peninsula (Rebelo et al., 2006).

2.2. Target species selection

In order to select species for plant functional diversity, ecosystem functioning and resilience (Clewell and Aronson, 2013; Diaz and Cadibo, 2001; Funk et al., 2008; Holmes and Richardson, 1999), a comprehensive list of species occurring within Peninsula Shale Renosterveld was compiled and arranged according to growth forms. For the purpose of this study, however, remnants were assessed to reduce the species list, from 668 species, to a more manageable number according to population location, population size and the likelihood of populations producing adequate seed within the time-frame identified. Although collection of as many species as possible is recommended for restoration of fynbos and other fire-adapted shrublands (Holmes and Richardson, 1999), it was decided that approximately 25–35 species would be appropriate for this study. Addressing the links between succession theory and restoration ecology, Del Moral et al. (2007) recommend selecting species from seral stages of the successional sequence in order to successfully direct the restoration trajectory. Ideally more Fabaceae species and fewer Asteraceae species could have been collected, however the legumes were very difficult and time-consuming to collect and, with the exception of *Podalyria sericea*, produced too little seed to include in this project. Seed was ultimately collected from 31 species, representing 14 families and six growth forms (Table 1).

2.3. Seed collection, processing and storage

Seeds from most species were collected from October 2011 to February 2012 with several species being collected in May 2012. The seed was temporarily kept in a well-ventilated room and fumigated with insecticide prior to storage at 15% RH and 15 °C for approximately 4 months; thereafter, the seed was cleaned.

2.4. Viability trial

A viability test is an indirect measure of germination potential that determines the proportion of seeds that are alive and theoretically capable of germinating (Gosling, 2003). As an initial method to estimating viability, four replicates of 25 seeds per species were x-rayed to ascertain seed fill, using a Faxitron digital X-ray machine (Qados, Sandhurst, U.K.), set at the standard Millennium Seed Bank settings (22 kV and 0.3 mA for 20 s). Once x-rayed, seeds were tested for viability; in this study viability is comprised of germinated seedlings plus seeds that failed to germinate but appeared internally healthy (white and structurally sound). The pre-treatments and parameters implemented in these viability trials largely attempted to mimic the conditions of the glasshouse seedling emergence trial; consequently, the methodology of these viability tests may differ from protocols previously carried out for the same, or similar, species (Brown and Botha, 2004; RBG Kew, 2009). Seed replicates were placed in dry Petri dishes over water in a sealed container for rehydration at 100% RH and 20 °C for 24 h to prevent imbibition damage during soaking pretreatments. Three species (*Arctopus echinatus*, *Myrsine africana* and *P. sericea*) were hot water treated in 250 ml water at 80 °C and allowed to cool. Thereafter, the

Table 1
Seed collected from 31 species representing six growth forms (five graminoids, five geophytes, three forbs, four succulent shrubs, 10 low shrubs and four tall shrubs).

Species	Family	Growth form
Arctopus echinatus	Apiaceae	Forb
Athanasia crithmifolia	Asteraceae	Tall shrub
Babiana fragrans	Iridaceae	Geophyte
Chironia baccifera	Gentianaceae	Low shrub
Chrysocoma coma-aurea	Asteraceae	Low shrub
Cymbopogon marginatus	Poaceae	Graminoid
Dimorphotheca pluvialis	Asteraceae	Forb (annual)
Ehrharta calycina	Poaceae	Graminoid
Erepsia anceps	Mesembryanthemaceae	Succulent shrub
Eriocephalus africanus var. *africanus*	Asteraceae	Low shrub
Felicia filifolia	Asteraceae	Low shrub
Helichrysum cymosum subsp. *cymosum*	Asteraceae	Low shrub
Helichrysum patulum	Asteraceae	Low shrub
Hermannia hyssopifolia	Malvaceae	Low shrub
Lachenalia fistulosa	Hyacinthaceae	Geophyte
Lampranthus emarginatus	Mesembryanthemaceae	Succulent shrub
Moraea bellendenii	Iridaceae	Geophyte
Myrsine africana	Myrsinaceae	Tall shrub
Ornithogalum thyrsoides	Hyacinthaceae	Geophyte
Othonna arborescens	Asteraceae	Succulent shrub
Pelargonium cucullatum subsp. *tabulare*	Geraniaceae	Low shrub
Pentameris airoides subsp. *airoides*	Poaceae	Graminoid (annual)
Podalyria sericea	Fabaceae	Low shrub
Ruschia rubricaulis	Mesembryanthemaceae	Succulent shrub
Salvia africana-caerulea	Lamiaceae	Low shrub
Searsia laevigata var. *villosa*	Anacardiaceae	Tall shrub
Searsia tomentosa	Anacardiaceae	Tall shrub
Tenaxia stricta	Poaceae	Graminoid
Themeda triandra	Poaceae	Graminoid
Trachyandra muricata	Asphodelaceae	Geophyte
Ursinia anthemoides subsp. *anthemoides*	Asteraceae	Forb (annual)

seeds were sterilized for 5 min in a 10% (v/v) Domestos solution, rinsed thoroughly in distilled water, soaked overnight for 18 h at 15 °C in Kirstenbosch smoke primer solution (one primer disc per 50 ml water) (SANBI, 2013a), plated on 1% (w/v) agar and placed in an incubator at alternating temperatures of 20/10 °C with lighting provided in the warm phase (8 h). Replicates were checked weekly and germinated seedlings removed from the plate and recorded. Germination was scored when the radicle was at least 2 mm long. After 12 weeks of no germination, or 4 weeks of no further germination, non-germinated seeds were dissected to determine the number of seeds that were 'healthy' (likely to be viable) or 'mouldy' (non-viable). If the majority of seeds were healthy on dissection, they were placed into a 1% (w/v) solution of 2,3,5-triphenyl tetrazolium chloride (TZ) for 72 h at 30 °C in the dark to identify the metabolically active seeds and the staining interpreted according to the Tetrazolium Testing Handbook (AOSA, SCST, 2010). Since no specific guidelines for the majority of species in this study are currently available, seeds were considered viable if the embryo and any living storage tissues stained a uniform red or dark red colour. The TZ results were not included in the viability calculation, but provide an explanation of why apparently viable seeds did not germinate. In this study, viability is calculated as:

$$\%\ \text{'viable' seeds} = (\text{no. germinated} + \text{no. healthy})/(\text{no. germinated} + \text{no. healthy} + \text{no. mouldy}) \times 100.$$

2.5. Seedling emergence trial

An emergence (or germination) test is a direct measure of the proportion of seeds capable of germinating into 'normal' healthy seedlings (Gosling, 2003). The same three species were hot-water treated as in the viability trial and five replicates of 25 seeds of each of the 31 species were sown into polystyrene trays at a rate of one seed per cavity (40 × 40 × 90 mm) and lightly covered with silica sand (0.25–1 mm). The substrate was composed of four parts compost (stable manure, chipped fynbos plant material and urea), three parts bark (6–12 mm decomposed pine bark), one part silica sand (0.25–1 mm) and 5% peat moss. The trays were smoked in a smoke tent, placed in the glasshouse and watered with fungicide additive (Apron XL with active ingredient mefenoxam, 1 ml/3 kg seed). Hand-watering was carried out approximately three times per week. Replicates were checked monthly and seedling emergence scored when seedlings were first visible.

2.6. Restoration species index

Based on a review of the literature and expert opinion, indicators relevant to the issues of species selection and seed collection were identified for incorporation into the index: seed source proximity to restoration site (Bautista et al., 2009; Hufford and Mazer, 2003); population size (Broadhurst et al., 2008; Hufford and Mazer, 2003; Kaye, 2001; Mijnsbrugge et al., 2010; Way, 2003); extent of area (Kaye, 2001; Mijnsbrugge et al., 2010; Way, 2003); plant abundance; seeds per individual; conservation status (Falk et al., 1996) (as classified in the Red List of South African Plants) (SANBI, 2012); and, percent seed viability and seedling emergence, as determined by the respective trials (Table 2). These indicators were divided into categories appropriate to the effect size of each indicator and values assigned to each category in order to rank each species within the index range. To emphasize indicators of greater importance to the identified issues, some indicators were weighted in the order of two. Data for the seed collection indicators were estimates derived solely from the areas in which seed was harvested for this project and may not be representative of other areas within the ecosystem or the ecosystem as a whole.

2.7. Limitations

We acknowledge the limitation of populating the restoration species index with seedling emergence results from the glasshouse trial. A next logical step would be to test the index in the field. In addition, the seed lots tested in the quality tests were from a single seed production event and may not account for variability in seed quality produced among years.

2.8. Statistical analysis

To determine differences between seed viability and seedling emergence, two-sample, one-sided binomial tests were carried out [Genstat statistical package 15th edition (VSN International Ltd., 2012)]. In order to identify restoration potential for each of the 31 species, the possible restoration species index range (from scores six to 49) was divided into three categories of restoration potential: low (<21), moderate (21–35) and high (>35).

3. Results

3.1. Seed viability and seedling emergence

For 22 of the 31 species (approximately 71%), seed viability was significantly greater than seedling emergence. Fifteen of the 31 species (approximately 48%) had seedling emergence less than 50%. Seven of the 31 species (approximately 23%) were classified as non-problematic as seed viability was not statistically greater than seedling emergence and emergence was in excess of 50%. These species were *Athanasia crithmifolia*, *Babiana fragrans*, *Chironia baccifera*, *Eriocephalus africanus* var. *africanus*, *Helichrysum patulum*, *P. sericea* and *Salvia africana-caerulea* (Table 3).

Table 2
Restoration species index key, comprised of indicators divided into categories numerically scored from zero to eight. High values represent high restoration potential.

Score	(0)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
Proximity to restoration site		Remnant 7 km from site	Remnant 7 km from site and adjacent remnant	Adjacent remnant					
Population size		<250	250–1000	1000–5000	5000–10,000	10,000–20,000	20,000–30,000	>30,000	
Extent of area (ha)		<0.25	0.25–0.5	0.5–2.5	2.5–5	5–7.5	7.5–10	>10	
Plant abundance		Rare	Occasional	Frequent	Common	Abundant			
Seeds per individual		<10	10–50	50–100	100–250	250–500	500–1000	>1000	
Conservation status		Least concern			Conservation concern				
Emergence (%)	0		0–25		25–50		50–75		75–100
Viability (%)	0		0–25		25–50		50–75		75–100

3.2. Restoration species index

Of the 31 species, 3 species (approximately 10%) occurred in the low category (scoring <21), 21 species (approximately 68%) occurred in the moderate category (scoring 21–35) whilst seven species (approximately 22%) occurred in the high category (scoring >35) (Table 4).

4. Discussion

This paper makes an empirical contribution to the field of ecological restoration by supplementing seed physiological data for 31 renosterveld species, and a theoretical contribution in proposing an index to identify suitable restoration species towards minimising impact on seed source populations and promoting restoration risk mitigation, success and cost-effectiveness.

There is considerable value in comparing the methods and outcomes of the different seed quality tests, as the factors underlying discrepancies can be identified. Through such comparisons one can build up a cross-referenced repository for each species with respect to the effects of pretreatments and parameters on species performance, ultimately to identify, among species, the optimal germination cues required for high yields. The accumulation of seed physiological information of this nature has several useful implications for restoration practice (Implications for practice box).

4.1. Comparison of seed viability with seedling emergence

Viability levels were generally high, indicating seed of high quality and germination potential. However, almost three quarters of the species exhibited seedling emergence significantly lower than viability, indicating sub-optimal germination cues and environmental parameters. Seedlings may germinate but not emerge from the substrate for several reasons and different conditions in the glasshouse emergence trial compared with those in the laboratory viability trial are likely to

Table 3
Summary results for viability, seedling emergence and binomial test significance. Viability (%) ± SE: $n = 100$, except where indicated in brackets. Seedling emergence (%) ± SE: $n = 125$. In the emergence trial, the number of *Eriocephalus africanus* var. *africanus* seeds was not known, as four replicates of 25 fruit were sown and since 100 fruit cleaned for the viability trial revealed 218 seeds, in the emergence trial n was almost certainly greater than 125 (†). For four species, seedling emergence exceeded viability, most likely due to sampling error (‡). Significance: * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$.

Species	Growth form	Viability $n = 100$, except where indicated in brackets		Emergence $n = 125$	Significance
		Mean % ± SE	(n)	Mean % ± SE	p value
Arctopus echinatus	Forb	27.9 ± 13.61	(101)	6.4 ± 1.60	<0.001***
Athanasia crithmifolia	Tall shrub	64.3 ± 2.84	(98)	53.6 ± 6.01	0.054
Babiana fragrans	Geophyte	95.0 ± 3.00‡		99.2 ± 0.80	0.974
Chironia baccifera	Low shrub	75.3 ± 4.38	(101)	67.2 ± 9.50	0.093
Chrysocoma coma-aurea	Low shrub	93.8 ± 1.16	(97)	84.8 ± 4.08	0.018*
Cymbopogon marginatus	Graminoid	98.0 ± 1.18	(98)	76.0 ± 4.00	<0.001***
Dimorphotheca pluvialis	Forb (annual)	88.0 ± 2.31		4.0 ± 1.79	<0.001***
Ehrharta calycina	Graminoid	96.0 ± 1.63		85.6 ± 4.31	0.005**
Erepsia anceps	Succulent shrub	13.0 ± 5.00‡		28.0 ± 4.73	0.997
Eriocephalus africanus var. *africanus*	Low shrub	46.3 ± 1.91‡	(218)	62.4 ± 3.49†	0.998
Felicia filifolia	Low shrub	79.8 ± 2.84	(99)	57.6 ± 2.04	<0.001***
Helichrysum cymosum subsp. *cymosum*	Low shrub	88.7 ± 3.95	(96)	70.4 ± 4.66	<0.001***
Helichrysum patulum	Low shrub	81.6 ± 7.14	(98)	75.2 ± 5.57	0.125
Hermannia hyssopifolia	Low shrub	61.6 ± 4.25	(99)	0.0 ± 0.00	<0.001***
Lachenalia fistulosa	Geophyte	96.8 ± 1.96	(96)	51.2 ± 3.20	<0.001***
Lampranthus emarginatus	Succulent shrub	92.9 ± 2.02	(99)	22.4 ± 5.60	<0.001***
Moraea bellendenii	Geophyte	98.0 ± 2.58	(97)	84.8 ± 4.08	<0.001***
Myrsine africana	Tall shrub	93.0 ± 3.00		25.6 ± 2.04	<0.001***
Ornithogalum thyrsoides	Geophyte	98.0 ± 1.15		77.6 ± 2.04	<0.001***
Othonna arborescens	Succulent shrub	80.0 ± 2.31		64.8 ± 3.44	0.006**
Pelargonium cucullatum subsp. *tabulare*	Low shrub	84.0 ± 6.73		7.2 ± 1.50	<0.001***
Pentameris airoides subsp. *airoides*	Graminoid (annual)	53.8 ± 5.01	(95)	0.0 ± 0.00	<0.001***
Podalyria sericea	Low shrub	79.0 ± 5.97‡		89.6 ± 2.40	0.986
Ruschia rubricaulis	Succulent shrub	83.0 ± 9.15		0.0 ± 0.00	<0.001***
Salvia africana-caerulea	Low shrub	59.5 ± 8.66	(99)	52.8 ± 5.85	0.155
Searsia laevigata var. *villosa*	Tall shrub	1.0 ± 1.00		0.0 ± 0.00	0.131
Searsia tomentosa	Tall shrub	5.0 ± 1.91	(98)	0.0 ± 0.00	0.005**
Tenaxia stricta	Graminoid	91.8 ± 1.61	(97)	20.0 ± 2.19	<0.001***
Themeda triandra	Graminoid	46.0 ± 14.65		29.6 ± 2.04	0.006**
Trachyandra muricata	Geophyte	98.0 ± 1.15		11.2 ± 2.65	<0.001***
Ursinia anthemoides subsp. *anthemoides*	Forb (annual)	79.0 ± 9.15		36.8 ± 4.27	<0.001 ***

Table 4
Numerical score per indicator, total score and category of restoration potential (low/moderate/high) for each of the 31 species.

Species	Growth form	Proximity to restoration site	Population size	Extent of area	Plant abundance	Seed abundance (per individual)	Conservation status	Emergence	Viability	Total score	Low/ moderate/ high
Arctopus echinatus	Forb	1	1	4	2	1	1	2	4	16	L
Athanasia crithmifolia	Tall shrub	3	7	4	5	7	1	6	6	39	H
Babiana fragrans	Geophyte	1	7	7	4	3	4	8	8	42	H
Chironia baccifera	Low shrub	1	1	3	3	7	1	6	8	30	M
Chrysocoma coma-aurea	Low shrub	3	5	3	4	7	1	8	8	39	H
Cymbopogon marginatus	Graminoid	2	5	4	3	4	1	8	8	35	H
Dimorphotheca pluvialis	Forb (annual)	1	3	2	2	2	1	2	8	21	M
Ehrharta calycina	Graminoid	1	2	3	2	2	1	8	8	27	M
Erepsia anceps	Succulent shrub	2	3	3	3	4	1	4	2	22	M
Eriocephalus africanus var. *africanus*	Low shrub	1	3	3	3	2	1	6	4	23	M
Felicia filifolia	Low shrub	1	2	3	3	6	1	6	8	30	M
Helichrysum cymosum subsp. *cymosum*	Low shrub	1	2	4	2	4	1	6	8	28	M
Helichrysum patulum	Low shrub	1	1	3	2	4	1	8	8	28	M
Hermannia hyssopifolia	Low shrub	2	7	6	4	2	1	0	6	28	M
Lachenalia fistulosa	Geophyte	1	7	7	4	4	1	6	8	38	H
Lampranthus emarginatus	Succulent shrub	1	2	1	2	4	1	2	8	21	M
Moraea bellendenii	Geophyte	1	1	3	1	2	1	8	8	25	M
Myrsine africana	Tall shrub	3	1	3	2	3	1	4	8	25	M
Ornithogalum thyrsoides	Geophyte	1	7	3	5	4	1	8	8	37	H
Othonna arborescens	Succulent shrub	1	4	3	3	2	1	6	8	28	M
Pelargonium cucullatum subsp. *tabulare*	Low shrub	2	3	3	3	3	1	2	8	25	M
Pentameris airoides subsp. *airoides*	Graminoid (annual)	1	5	3	3	3	1	0	6	22	M
Podalyria sericea	Low shrub	1	3	2	4	4	4	8	8	34	M
Ruschia rubricaulis	Succulent shrub	1	3	2	3	6	4	0	8	27	M
Salvia africana-caerulea	Low shrub	2	5	7	3	4	1	6	6	34	M
Searsia laevigata var. *villosa*	Tall shrub	2	1	2	2	7	1	0	2	17	L
Searsia tomentosa	Tall shrub	3	2	3	4	7	1	0	2	22	M
Tenaxia stricta	Graminoid	1	3	2	2	4	1	2	8	23	M
Themeda triandra	Graminoid	1	3	3	2	2	1	4	4	20	L
Trachyandra muricata	Geophyte	1	7	7	4	6	1	2	8	36	H
Ursinia anthemoides subsp. *anthemoides*	Forb (annual)	1	3	1	3	3	1	4	8	24	M

account for observed differences in emergence and germination respectively; these include water availability (hand-watering versus continual moisture supply); temperature (variable versus incubator-controlled); light (no-low versus direct); substrate (soil mix versus 1% agar); and smoke (smoke versus smoke extract solution containing, *inter alia*, gibberellic acid). Since germination of many fynbos species, a point of reference as the better-studied adjacent vegetation type to renosterveld, results from the diurnal temperature fluctuation of autumn (Holmes, 2002b), sowing the seedling emergence trials marginally later, in May 2012, may have adversely affected germination in general.

4.2. Seed viability significantly greater than seedling emergence

Each of the 22 species with viability significantly greater than emergence is briefly discussed below.

Four of the 22 species had zero seedling emergence. As seed coat impermeability occurs in some Malvaceae species (Dickie and Stuppy, 2003), after 11 weeks of no germination in *Hermannia hyssopifolia*, a small portion of the seed coat was removed with a scalpel in the laboratory trial only, and this difference in pre-treatments is almost certainly responsible for the discrepancy in performances. For *Searsia tomentosa*, both viability and emergence were low and an X-ray of the sample confirmed the expectation of a large proportion of infested (endophagous insects present) and partially-filled seeds (Newton, 2012). *Pentameris airoides* subsp. *airoides* is most likely sensitive to environmental variables as viability was moderate despite an X-ray of the sample indicating almost all seeds were fully-formed and seedlings failed to emerge in the glasshouse, even though trial pre-treatments were comparable. The majority of *Ruschia rubricaulis* seeds were healthy on dissection, however, the embryos stained poorly in TZ. The harvested seed capsules had been produced the preceding season, thus excluding old seed as a possible explanation and instead, poor genetic fitness of the single, small, isolated population from which they were harvested may explain the lack of emergence and poor TZ results.

Poor TZ staining in several species may simply have been due to low seed quality. The harvested *Lampranthus emarginatus* capsules had been produced two seasons previously and it is more likely that deteriorated seed, as opposed to poor genetic fitness, was responsible for its poor emergence, as supported by the X-ray indicating a large proportion of filled seeds. Although the seeds of *Dimorphotheca pluvialis* stained deep red, this result may be indicative of extensive bruising (AOSA, SCST, 2010). The X-ray of *A. echinatus* confirmed a very high proportion of the seeds were partially-filled and although of ethnobotanical importance (Magee et al., 2008), the species is horticulturally insignificant and there is currently no information regarding its cultivation (SANBI, 2013b).

Alternatively, poor TZ staining in species (e.g. *R. rubricaulis*, *L. emarginatus*, *M. africana*, *Themeda triandra*, *Trachyandra muricata* and *Ursinia anthemoides* subsp. *anthemoides*) may have been due to seed composition, as TZ does not easily permeate oily seeds (Wood et al., 2005) or deeply dormant seeds (e.g. *U. anthemoides* (Schutz et al., 2002)) which require longer staining times, higher solution concentrations and special preconditioning treatments to promote proper TZ staining (AOSA, SCST, 2010).

In other species, high viability is supported by moderate to good TZ staining and thus relatively low emergence in these species is probably due to environmental conditions failing to stimulate germination or inhibiting emergence. *Pelargonium* seeds are hard-coated (Holmes and Newton, 2004) and germination (and emergence) would almost certainly have improved by chipping the seed coat (Brown and Botha, 2004; RBG Kew, 2009). Graminoids, e.g. *Tenaxia stricta*, as with the annual taxa, require good drainage and the soil mix used in the emergence trial may have adversely affected these performances (A Hitchcock 2013, SANBI, personal communication). The viability of *T. muricata* dropped by almost a third when interpreting the TZ results yet still far exceeded emergence, suggesting sub-optimal conditions for germination and emergence. Although *Trachyandra* species are not currently cultivated, cultivation is not expected to be problematic (SANBI, 2013b). Despite seedling emergence being significantly lower than viability, species with moderate to high emergence, and thus raising little concern, include *Chrysocoma coma-aurea*, *Cymbopogon marginatus*, *Ehrharta calycina*, *Felicia filifolia*, *Helichrysum cymosum* subsp. *cymosum*, *Lachenalia fistulosa*, *Moraea bellendenii*, *Othonna arborescens* and *Ornithogalum thyrsoides*. The performance discrepancies for these species are largely attributable to variable environmental parameters as, of these species, only the *L. fistulosa* seeds were pre-treated differently, the seeds being chipped in the viability trial only.

4.3. Seedling emergence less than 50%

Seedling emergence outcomes were variable among species and almost half of the species failed to achieve emergence in excess of 50%. For *Erepsia anceps* and *Searsia laevigata* var. *villosa*, poor viability and emergence are indicative of low quality seed. As with *L. emarginatus*, the poor seed quality of *E. anceps* is most likely due to deterioration over time whilst that of *S. laevigata* var. *villosa* is attributable to the high number of partially-filled seeds evident in the X-rays. The remaining 13 species with emergence less than 50%, exhibited emergence significantly lower than viability, and have already been discussed above.

4.4. Non-problematic species

Almost a quarter of the species were non-problematic as, among species, the relationship between viability and emergence was not significantly different, affirming that dormancy had been effectively alleviated and environmental parameters were suitable, and emergence was in excess of 50%, indicative of likely good in-field performance. These species represent three of the six growth forms: geophytes, low shrubs and tall shrubs.

The variable outcomes from the seed physiology tests emphasize the importance of ascertaining seed quality and optimal environmental parameters and germination cues prior to embarking on large-scale seed collection and restoration. Scrutiny of the factors underpinning large discrepancies between viability, which was generally high, and emergence, highlights the roles of dormancy alleviation and optimal environmental conditions in achieving germination success. The value of understanding seed physiology and how this information can improve restoration efforts is clearly demonstrated. In a few instances, where little is known about a taxon, findings point to a need for further experimentation, testing different pre-treatments under a range of conditions.

4.5. Restoration species index

The vast majority of species occurred in the moderate to high index range indicating good potential for use in future seed-based restoration efforts. Growth forms, deemed critical for long-term ecological functioning (Diaz and Cadibo, 2001; Holmes and Richardson, 1999), were represented across the categories with the exception of the forbs, which only occurred in the low and moderate categories, and the low shrubs and geophytes, which only occurred in the moderate and high categories. In the moderate and high index range, species from all 14 families were represented, with the exception of Anacardiaceae and Apiaceae.

The restoration species index draws together a number of suggested criteria into a single approach in the challenging space of species selection. It allows for the rapid sorting of species at the outset and gives a clear representation of the value of species, demonstrating the practical relevance of the proposed index for future use in mitigating some of the high time, cost and in-field performance risk factors associated with any ecological restoration project (Crookes et al., 2013; Lippitt et al., 1994; Macmillan et al., 1998). In this study the index proved useful where the vast majority of species exhibited moderate to high potential, and in addition, it pulled the poor-performing species into focus, prompting consideration of the trade-off between collection-time in the field versus likely reward with respect to how desirable the species is for the future community.

Although the index presented here is particular to just 31 renosterveld species, it may be readily tailored with individualised weighting so as to evaluate species with the traits appropriate for addressing project-specific criteria, for instance rainfall reliability (Fenner and Thompson, 2005) or the ability to colonize, compete and regenerate (Pywell et al., 2003). Another application could be to identify species sharing traits with likely invasive species in order build invasion resistance through niche preoccupation (Funk et al., 2008). The index, whilst relevant to the specific parameters of this study, is a species selection tool that warrants expansion, further examination and testing in this and other ecosystems. One starting point might be to use it on older restoration studies, incorporating criteria reflecting longer-term in-field performance, to provide a more faithful measure of the usefulness of species and furthermore to see how it holds out over time.

5. Implications for practice box

Several practical benefits, derived from the restoration species index and knowledge of seed viability and/or seedling emergence, are central to informing efficient and responsible restoration protocols. These benefits include:

- Targeting likely high-yield species for seed collection and use;
- Adjusting the quantity of seed for collection based on knowledge of likely viability and/or emergence;
- Determining when to seek alternative approaches to seed reintroduction for problematic guilds (e.g. legumes) such as reintroducing species as nodes of planted material;
- Determining when to initiate further germination-cue experimentation under a range of environmental parameters;
- Promoting collections of genetic integrity yet adequate diversity;
- Ensuring source population resilience;
- Promoting seed collection ‘ease’ and cost-effectiveness; and,
- Informing the timing and methodology of restoration implementation on the basis of optimal germination cues and environmental parameters.

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References

AOSA, SCST (Association of Official Seed Analysts, Society of Commercial Seed Technologists), 2010. Tetrazolium Testing Handbook. AOSA, SCST, Ithaca, New York.

Bautista, S., Aronson, J., Vallejo, R.V., 2009. Land Restoration to Combat Desertification: Innovative Approaches, Quality Control and Project Evaluation. Fundación Centro de Estudios Ambientales del Mediterráneo, Spain.

Blakesley, D., Anusarnsunthorn, V., Kerby, J., Nnavakitbumrang, P., Kuarak, C., Zangkum, S., Hardwick, K., Elliot, S., 2000. Nursery technology and tree species selection for restoring forest biodiversity in northern Thailand. In: Elliott, S., Kerby, J., Blakesley, D., Hardwick, K., Woods, K., Anusarnsunthorn, V. (Eds.), Forest Restoration for Wildlife Conservation. International Timber Organisation and The Forest Restoration Research Unit, Chiang Mai University, Thailand, pp. 207–222.

Boucher, C., 1995. Overview of management of Renosterveld. In: Low, A.B., Jones, F.E. (Eds.), The Sustainable Use and Management of Renosterveld Remnants in the Cape Floristic Region. Proceedings From a Symposium. FCC Report 95/4. Flora Conservation Committee, Botanical Society of South Africa, Kirstenbosch, pp. 47–50.

Broadhurst, L.M., Lowe, A., Coates, D.J., Cunningham, S.A., McDonald, M., Vesk, P.A., Yates, C., 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. Evolutionary Applications 1, 587–597.

Brown, N.A.C., Botha, P.A., 2004. Smoke seed germination studies and a guide to seed propagation of plants from the major families of the Cape Floristic Region, South Africa. South African Journal of Botany 70, 559–581.

Clewell, A.F., Aronson, J., 2013. Ecological Restoration: Principles, Values, and Structure of an Emerging Profession. 2nd ed. Island Press, Washington.

Conservation International, 2013. Hotspots revisited. URL, http://www.conservation.org/ ([accessed 15 August 2013]).

Crookes, D.J., Blignaut, J.N., de Wit, M.P., Esler, K.J., le Maître, D.C., Milton, S.J., Mitchell, S.A., Cloete, J., de Abreu, P., Fourie (nee Vlok), H., Gull, K., Marx, D., Mugido, W., Ndhlovu, T., Nowell, M., Pauw, M., Rebelo, A., 2013. System dynamic modelling to assess economic viability and risk trade-offs for ecological restoration in South Africa. Journal of Environmental Management 120, 138–147.

Del Moral, R., Walker, L.R., Bakker, J.P., 2007. Insights gained from succession for the restoration of landscape structure and function. In: Walker, L.R., Walker, J., Hobbs, R.J. (Eds.), Linking Restoration and Ecological Restoration. Springer Science, New York.

Diaz, S., Cadibo, M., 2001. Vive la Différence: Plant Functional Diversity Matters to Ecosystem Processes 16. Elsevier Science, pp. 646–655.

Dickie, J.B., Stuppy, W.H., 2003. Seed and fruit structure. In: Smith, R.D., Dickie, J.B., Linington, S.H., Prichard, H.W., Probert, R.J. (Eds.), Seed Conservation: Turning Science Into Practice. Royal Botanic Gardens, Kew, pp. 255–279.

Elliott, S., Navakitbumrung, P., Kuarak, C., Zangkum, S., Anusarnsunthorn, V., Blakesley, D., 2003. Selecting framework tree species for restoring seasonally dry tropical forests in northern Thailand based on field performance. Forest Ecology and Management 184, 177–191.

Elliott, S., Blakesley, D., Hardwick, K., 2013. Restoring Tropical Forests: A Practical Guide. Royal Botanic Gardens, Kew, p. 344.

Falk, D.A., Millar, C.I., Olwell, M., 1996. Restoring Diversity: Strategies for Reintroduction of Endangered Plants. Island Press, Washington DC.

Fenner, M., Thompson, K., 2005. The Ecology of Seeds. Cambridge University Press.

Funk, J.L., Cleland, E.E., Suding, K.N., Zavaleta, E.S., 2008. Restoration through reassembly: plant traits and invasion resistance. Trends in Ecology and Evolution 23, 695–703.

Goosem, S.P., Tucker, N.I.J., 1995. Repairing the Rainforest — Theory and Practice of Rainforest Re-establishment in North Queensland's Wet Topics. Wet Tropics Management Authority, Cairns.

Gosling, P., 2003. Viability testing. In: Smith, R.D., Dickie, J.B., Linington, S.H., Prichard, H.W., Probert, R.J. (Eds.), Seed Conservation: Turning Science Into Practice. Royal Botanic Gardens, Kew, pp. 445–481.

Grime, J.P., 1979. Plant Strategies and Vegetation Processes. Wiley, Chichester.

Helme, N.A., Rebelo, A.B., 2005. Renosterveld ecosystems. In: De Villiers, C.C., Driver, A., Clark, B., Euston-Brown, D.I.W., Day, E.G., Job, N., Helme, N.A., Holmes, P.M., Brownlie, S., Rebelo, A.B. (Eds.), Ecosystem Guidelines for Environmental Assessment in the Western Cape. Fynbos Forum and Botanical Society of South Africa Conservation Unit, Cape Town.

Holmes, P.M., 2002a. Renosterveld restoration on cultivated lands at Eerste River, Western Cape: preliminary findings of a pilot study. Unpublished report, Lafarge Quarries.

Holmes, P.M., 2002b. Depth distribution and composition of seed-banks in alien-invaded and uninvaded fynbos vegetation. Austral Ecology 27, 110–120.

Holmes, P.M., 2005. Results of a lucerne old-field restoration experiment at the Fynbos–Karoo interface. South African Journal of Botany 71, 326–338.

Holmes, P.M., Newton, R.J., 2004. Patterns of seed persistence in South African Fynbos. Plant Ecology 172, 143–158.

Holmes, P.M., Richardson, D.M., 1999. Protocols for restoration based on recruitment dynamics, community structure, and ecosystem function: perspectives from South African fynbos. Restoration Ecology 7, 215–230.

Hufford, K.M., Mazer, S.J., 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends in Ecology and Evolution 18, 147–155.

Kaye, T.N., 2001. Common ground and controversy in native plant restoration: the SOMS debate, source distance, plant selections, and a restoration oriented definition of native. In: Haase, D.L., Rose, R. (Eds.), Proceedings: Native Plant Propagation and Restoration Strategies. Nursery Technology Cooperative and Western Forestry and Conservation Association, Oregon, pp. 5–12.

Knowles, O.H., Parrotta, J.A., 1995. Amazonian forest restoration: an innovative system for native species selection based on phenological data and field performance indices. Commonwealth Forestry Review 74, 230–243.

Lamb, D., Parrotta, J.A., Keenan, R., Tucker, N.I.J., 1997. Rejoining habitat remnants: restoring degraded rainforest lands. In: Laurence, W.F., Bierregaard Jr., R.O. (Eds.), Tropical Forest Remnants: Ecology, Management and Conservation of Fragmented Communities. University of Chicago Press, pp. 366–385.

Lippitt, L., Fidelibus, M.W., Bainbridge, D.A., 1994. Native seed collection, processing, and storage for revegetation projects in the Western United States. Restoration Ecology 2, 120–131.

Macmillan, D.C., Harley, D., Morrison, R., 1998. Cost-effectiveness analysis of woodland ecosystem restoration. Ecological Economics 27, 313–324.

Magee, A.R., van Wyk, B.E., Tilney, P.M., van der Bank, M., 2008. A taxonomic revision of the South African endemic genus *Arctopus* (Apiaceae, Saniculoideae). Annals of the Missouri Botanical Garden 95, 471–486.

Manning, J., Goldblatt, P., 2012. Plants of the Greater Cape Floristic Region 1: The Core Cape Flora. Strelitzia 29. South African National Biodiversity Institute, Pretoria.

Midoko-Iponga, D., 2004. Renosterveld Restoration: The Role of Competition, Herbivory and Other Disturbances. MSc Thesis. University of Stellenbosch.

Mijnsbrugge, K.V., Biscoff, A., Smith, B., 2010. A question of origin: where and how to collect seed for ecological restoration. Basic and Applied Ecology 11, 300–311.

Milton, S.J., 2007. Rapid assessment method for Renosterveld. Renosterveld assessment workshop, unpublished report.

Newton, R.J., 2012. Report on seed germination of four Searsia species from SANParks. Unpublished report, Royal Botanic Gardens, Kew.

Pywell, R.F., Bullock, J.M., Roy, D.B., Warman, L., Walker, K.J., Rothery, P., 2003. Plant traits as predictors of performance in ecological restoration. Journal of Applied Ecology 40, 65–77.

RBG (Royal Botanic Gardens) Kew, 2009. Seed Information Database (SID). URL, http://data.kew.org/sid/sidsearch.html ([accessed 24 March 2012 and 13 March 2013]).

Rebelo, A.G., 1995. Renosterveld: conservation and research. In: Low, A.B., Jones, F.E. (Eds.), The Sustainable Use and Management of Renosterveld Remnants in the Cape Floristic Region. Proceedings From a Symposium. FCC Report 95/4. Flora Conservation Committee, Botanical Society of South Africa, Kirstenbosch, pp. 32–42.

Rebelo, A.G., Boucher, C., Helme, N., Mucina, L., Rutherford, M.C., 2006. Fynbos Biome. In: Mucina, L., Rutherford, M.C. (Eds.), The Vegetation of South Africa, Lesotho and Swaziland. South African National Biodiversity Institute, Pretoria, pp. 54–183.

SANBI (South African National Biodiversity Institute), 2012. Statistics: Red List of South African Plants Version 2013 ([accessed 10 August 2012]).

SANBI (South African National Biodiversity Institute), 2013a. Seed sales and Kirstenbosch Seedroom. URL, http://www.sanbi.org/gardens/kirstenbosch/kirstenbosch-nbg-seedroom ([accessed 13 March 2013]).

SANBI (South African National Biodiversity Institute), 2013b. Plantzafrica. URL, http://www.plantzafrica.com/index.html ([accessed 24 March 2012 and 13 March 2013]).

Schutz, W., Milberg, P., Lamont, B.B., 2002. Seed dormancy, after-ripening and light requirements of four annual Asteraceae in South-western Australia. Annals of Botany 90, 707–714.

Taylor, H.C., 1980. Phytogeography of fynbos. Bothalia 13, 231–235.

Von Hase, A., Rouget, M., Maze, K., Helme, N., 2003. A Fine-scale Conservation Plan for the Cape Lowlands Renosterveld: Technical Report. Cape Conservation Unit, Botanical Society, Cape Town.

VSN International Ltd., 2012. GenStat Statistical Package. 15th ed. Hemel Hempstead, UK.

Way, M.J., 2003. Collecting seed from non-domesticated plants for long-term conservation. In: Smith, R.D., Dickie, J.B., Linington, S.H., Prichard, H.W., Probert, R.J. (Eds.), Seed Conservation: Turning Science Into Practice. Royal Botanic Gardens, Kew, pp. 163–201.

Wood, C.B., Miles, S., Rix, C., Terry, J., Daws, M.I., 2005. The effect of seed oil content on viability assessment using tetrazolium: a case study using 171 species. Plant Genetic Resources, Newsletter 143, 17–23.